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The potential environmental impact of engineered nanomaterials

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With the increased presence of nanomaterials in commercial products, a growing public debate is emerging on whether the environmental and social costs of nanotechnology outweigh its many benefits. To date, few studies have investigated the toxicological and environmental effects of direct and indirect exposure to nanomaterials and no clear guidelines exist to quantify these effects.

From bacteria that sense the earth's magnetic field using 'nanomagnets,' to the facilitated transport of radionuclides in groundwater, nanoparticles are central to many natural processes[1–5]. The behavior of these naturally occurring materials results from their physical size, tunable properties and large and accessible inorganic surfaces. These same features can be optimized in engineered nanoparticles tailored for the requirements of diverse technologies, as illustrated elsewhere in this issue. However, as society begins to use nanomaterials in greater quantities and in consumer products, interest in the broader implications of this emerging technology has grown. The central question is whether the unknown risks of engineered nanoparticles, in particular their environmental impact, outweigh their established benefits for society.

How this debate evolves may be among the most important factors in defining the future trajectory of nanotechnology commercialization. Currently, most sectors of nanotechnology are developing with no regulation and in an environment ideally suited for entrepreneurship. This could change as environmental groups concerned about potential risks call for a moratorium on nanotechnology research and regulation of nanomaterials[6,7]. The lack of technical data on the topic provides fertile ground for both nanotechnology proponents and skeptics alike to make contradictory and sweeping conclusions about the safety of engineered nanoparticles. This atmosphere of uncertainty is precisely the feature of nanotechnology that causes skeptics the greatest concern. Their arguments have the attention of policymakers in Europe and the United States and could spawn nanotechnology-specific regulation[8–10]. This would transform the business and research enterprise of nanotechnology much as it did those of agricultural biotechnology. On the other hand, if the research community engages quickly to infuse technical data into this debate, the actual risks of engineered nanomaterials will become better defined. Such data will also provide the means to minimize environmental consequences well before a nanotechnology industry is established, leading to more successful and profitable technologies.

This commentary provides a technical introduction to the assessment of the environmental impact of engineered nanomaterials. It is a challenge to present such an analysis in the absence of a developed literature base. Much of the discussion is based on extrapolations from better understood molecular species and larger particulate matter. Also, it is far too premature to complete a formal risk assessment for engineered nanomaterials—in fact, it may never be possible with such a broad class of substances. However, the general framework of an assessment methodology is useful, even at this early stage, and provides structure to the following discussion. Thus, an analysis of the exposure routes for nanomaterials and their relative importance is presented separately from an overview of the known toxicology literature. Clearly, both issues will be equally important for characterizing the environmental risk of nanomaterials.

Nanoparticle exposure

How may people be exposed to engineered nanoparticles and in what quantities? All substances, from arsenic to table salt are toxic to cells, animals or people at some exposure level. Before interpreting toxicological data, it is thus essential to characterize the expected concentrations of engineered nanoparticles that may be present in the air, water and soil. A useful way to approach the problem is to consider how human populations, both in the present and near future, may be exposed to engineered nanoparticles. Each situation presents different issues for characterizing exposure, and their comparison highlights those scenarios most likely to be relevant for engineered nanomaterials. In this article, the phrase 'engineered nanomaterials' is used to describe inorganic materials of high uniformity, with at least one critical dimension below 100 nm, specifically engineered for applications.

The exposure of workers making and using nanoparticles in manufacturing plants is growing as the nanotechnology industry increases demand for small, size-controlled particles. Many new and established companies have announced the construction of plants devoted to producing nanoscale particles for diverse applications. For example, in May, Mitsubishi opened the first fullerene plant in Japan, which aims to produce tons of fullerenes this year alone for applications ranging from bowling ball coatings to fuel cells[11,12]. In the United States, the material safety data sheets (MSDS) for most nanomaterials lists properties and restrictions which are identical to those given for the bulk material. Thus, workers using these substances have no formal requirements for safety precautions beyond those adopted for bulk solids of identical composition.

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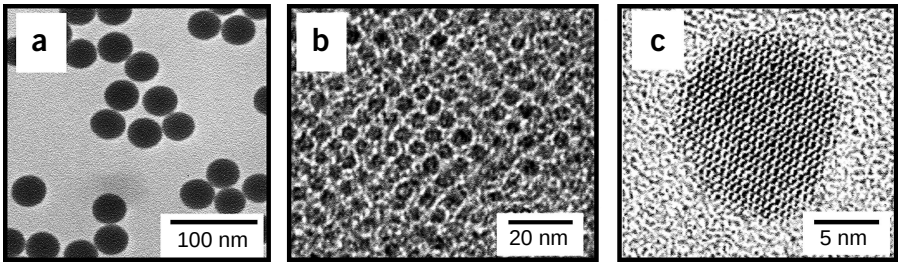

Figure 1 Transmission electron microscopy (TEM) of engineered nanoparticles. (**a**) Nanosized silica particles produced by the hydrolysis of silicon alkoxides. The high uniformity of these samples is a characteristic feature of many nanomaterials where control of size is necessary to define properties. (**b**) Nanocrystalline magnetite (Fe_3O_4). This close-packed array of nanocrystals, with an average diameter of 4 nm, was formed as a drop of dispersed nanocrystals dried onto a TEM support grid. The particles are not touching because of the presence of a surface passivating agent, oleic acid, which imparts solubility in nonpolar solvents. (**c**) High-resolution transmission electron micrograph of a single TiO_2 nanocrystal. The mottled background is the amorphous carbon support and the white and black dots represent columns of atoms throughout the anatase nanocrystal. The high crystalline perfection of this nanocrystal is a common feature of many engineered nanocrystals; because of their small size, defects formed during crystal growth can easily diffuse and anneal at nearby surfaces.

Occupational exposure to engineered nanoparticles presents a safety issue that superficially is familiar to both toxicologists and regulators alike. The term 'particle' has a distinct meaning to many in the worker safety community, and many workplace diseases result from chronic exposure to particulate matter[13,14]. Engineered nanomaterials (**Fig. 1**) differ in significant ways from the heterogeneous and polydisperse particulates traditionally associated with pulmonary toxicology. However, engineered nanomaterials (**Fig. 1**) differ in significant ways from ultrafine particles typically of interest in pulmonary toxicology. While ultrafine particles are defined as particles with diameters under 100 nm, their broad size distributions and heterogeneous compositions make comparisons with engineered nanomaterials problematic.

Though counterintuitive, it can be argued that inhalation exposures of engineered nanoparticles could be less significant than those observed either for ultrafine particulates or for larger micron-sized materials. Because UFPs are generated as aerosols, and exposure takes place in the same medium, the nanoscale particles have little chance to agglomerate in their dilute gaseous form. In contrast, as engineered nanomaterials are produced in liquid phase or in closed gas phase reactors, inhalation exposure would have to occur from the solid state. Because particle-particle interactions increase substantially in the nanoscale-size regime, engineered nanoparticles become strongly associated into bulk aggregates once removed from the liquid or gas phase. It is thus difficult to generate isolated and respirable nanoparticles from dried powders, though this can be altered by the appropriate control over surface coatings[15–17].

This reasoning could account for a recent unpublished study of the very low respirable levels of single-walled carbon nanotubes (SWNT) in a manufacturing facility. Because many engineered nanomaterials are prepared and ultimately processed in liquids, a more relevant exposure route for nanotechnology workers may be direct dermal absorption or oral ingestion.

Consumer exposure to engineered nanoparticles presents another exposure route for these materials. Engineered nanoparticles are used in personal care products, ranging from cosmetics to sunscreens, where decreasing the size of active ingredients, typically pigments, yields better performance[18–20]. It is impossible to assess the quantities and types of nanoparticles in such products as such information is often protected from public disclosure by trade secrets. Moreover, the US Food and Drug Administration (Rockville, MD, USA) ruled in 1999 that for sunscreens, 'micronized' titania was not a new ingredient, thus providing little incentive for toxicological studies of particle additives[21]. Existing literature rules out direct absorption of micronized titania (>40 nm diameters) through the skin[22,23]. A more important issue has been the stability of the organic components in sunscreens that contain nanoscale titania and zinc oxide particles. These materials are active photocatalysts, and the free radical species they generate under illumination can degrade sunscreen formulations[24–28]. These processes can also damage biological molecules, and although this poses some risk to consumers, it is generally not as significant as the risks of sun exposure[29,30]. Many of these reports rely on commercially available 'micronized' titania, and information relevant for particle sizes below 100 nanometers is not available.

The exposure of nanotechnology workers, and consumers using products containing engineered nanoparticles, is a near-term concern for most nanotechnology skeptics. Longer term, there is the opportunity for a much wider exposure of the entire ecosystem to engineered nanomaterials through the water and soil. Whether it is a zinc catalyst embedded in the rubber of a tire, or lead in household paint, the concentration of unnatural substances in the environment increases in direct proportion to their use in society[31]. If engineered-nanomaterial applications develop as projected, the increasing concentrations of nanomaterials in groundwater and soil may present the most significant exposure avenues for assessing environmental risk[32].

The first step in approaching this particular exposure route is to characterize the form and concentration of engineered nanomaterials in water. It is in water that many substances have their most significant environmental effects; even if only sparingly soluble, materials present in water can become degraded, transformed and accumulated in a variety of ways. Given the difficulty of creating water-soluble nanostructures in the laboratory for technologies, one might expect that engineered nanoparticles in most forms would not enter groundwater. This perception is not necessarily correct, however. C_{60} (fullerene) is a hydrophobic nanomaterial, but even when not surface treated it can form a stable colloidal species in water from both a powder and an organic solution (**Fig. 2**)[33–36].

For nanotechnology applications, the overall concentration of fullerenes in this colloidal form is too low (~10 p.p.m.) to be relevant for applications; for environmental scientists, this concentration is over a 100-fold the aqueous solubility limit of polyaromatic hydrocarbons (PAHs). Even at low aqueous concentrations, PAHs can have pronounced environmental effects, and fullerenes may have similar properties. This example illustrates the need to evaluate the physical form of engineered nanoparticles in groundwater and quantify their stability and concentration.

Nanomaterial health effects

Information about nanoparticle exposure conditions is only useful when paired with characterization of nanomaterial biological effects. This term refers broadly to data that can include experiments ranging from *in vitro* cellular toxicology to more traditional measurements of the lethal dose (LD_{50}; statistically derived dose of a

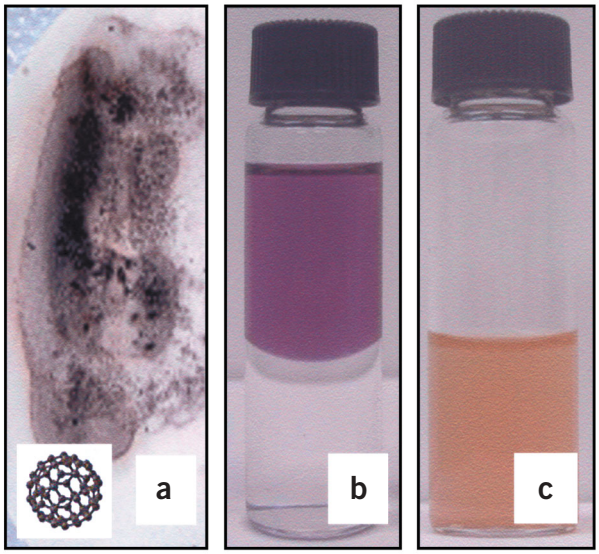

Figure 2 The diverse formats of engineered nanomaterials. (**a**) C_{60} dried onto filter paper is a black powder (inset: molecular structure of C_{60}). (**b**) Fullerenes are easily dissolved in nonpolar solvents, such as toluene, and form a purple solution (top layer). (**c**) With relatively mild chemical treatments, such as evaporation of the nonpolar phase, some C_{60} becomes water stable in this yellow solution. Although chemical analysis shows the presence of C_{60}, light scattering and electron microscopy confirm that the material is present as colloidal aggregates that contain between 100 and 1,000 fullerene molecules.

chemical/physical agent expected to kill 50% of organisms in a given population) of substances in animals. Because of the relative lack of data on engineered nanoparticles, any treatment of this topic must necessarily rely on a patchwork of distantly related information from pulmonary particle toxicology to biomaterial compatibility studies. More specific information concerning nanoparticle clearance and bioavailability can be gleaned from studies aiming to develop nanomaterials for biotechnology[37]. Finally, at least for carbon nanostructures, there are now a handful of papers that aim specifically to characterize the toxicology of these new materials.

Pulmonary toxicology is a well-developed area of medical research; many occupational diseases, such as asbestosis and coal miner's disease, are caused by the inhalation of inorganic particulate matter[13]. Recently, interest in the nanoscale particulate regime—defined as UFP by pulmonary toxicologists—has increased substantially[38–42]. Most reports find that UFPs are more toxic than equivalent micron-sized materials at similar doses per gram of body weight[43,44]; particle composition and surface chemistry, however, may be even more important to toxicological properties than size[45,46]. It is unlikely that these conclusions will translate directly to engineered nanomaterials. UFPs are chemically heterogeneous, polydisperse materials that bear little resemblance beyond their physical size to most engineered nanoparticles (**Fig. 1**)[47].

Despite the differences in materials, there is much that the emerging field of nanomaterial toxicology can learn from pulmonary toxicologists. Protocols for quantifying the dose-response effects of inorganic particulate matter have been developed for both *in vitro* and *in vivo* toxicological studies[48,49]. Also, well-characterized samples, such as micron-sized Suprasil quartz, provide a much needed benchmark for nanotoxicology. Using such materials as both positive and negative controls allows data from less predictive, but more practical, *in vitro* cell culture studies to be bridged to animal studies[50].

Particulate matter effects have also become important in the evaluation of biomaterial implants, such as those used for hip replacement; this literature highlights how protein adsorption may influence the toxicological response to particles[51–53]. It is well-established that wear processes cause implanted biomaterials to produce particles, ranging in size from tenths to several microns composed of either hydroxyapatite or the bulk replacement material (metals or polymers)[54–58]. These particles under some circumstances are cytotoxic and produce inflammatory responses that in some individuals can progress to bone loss[59–63]. The mechanism for these reactions is not clear. However, particle surfaces may provide sites for nonspecific protein adsorption. Immune responses raised against the particles would also recognize the native proteins. Specific surface treatment that eliminates protein adsorption would be an important method for minimizing such a process for engineered nanomaterials.

Because of the great interest in using engineered nanomaterials for medical applications, there is some information relevant for assessing health effects incidentally reported in this area of literature. In particular, the facile transport and association of engineered nanostructures with cells has received much attention from environmental groups who mistakenly interpret this feature as an indicator for toxicity. Water-soluble fullerenes, for example, associate strongly with cell membranes due to their hydrophobic nature[64–66]. This fact alone does not lead to an unusual toxic response and in fact is central to the use of these materials as antioxidants. Other engineered nanoparticles, such as quantum dots, that are appropriately derivatized can be taken into the cytoplasm of eukaryotes, presumably by receptor-mediated endocytosis[67,68]. Most cells have the ability to take up materials of a wide variety of sizes and shapes through receptor-mediated endocytosis or other processes. As the endosomes produced by these events can be 50 nm in diameter or larger, it is unclear to what extent cellular uptake is controlled by the physical size of the particles. Although this process is important to characterize because it alters the ways in which nanoparticles can influence biological processes, the observation alone does not strongly predict acute toxicity.

Carbon-nanostructures are the only class of engineered nanomaterials (as opposed to ultrafine particulates generated in aerosols) that have received focused toxicological characterization. Water-soluble forms of fullerenes have many promising medical applications based on their unique free-radical chemistry and antioxidant behavior[69–72]. As a result, many different forms of water-soluble fullerenes have been screened *in vitro* and in animal studies for toxicology. In cell culture, certain forms of water-soluble C_{60} are phototoxic (**Table 1**)[73–75].

Table 1 Summary of cytotoxicology data on water-soluble fullerenes

C_{60} type	Cell type	LC_{50}[a]	Notes	References
Polymer wrapped	Mouse fibroblasts	300 p.p.m.	Uncontrolled light	73
	Mouse fibroblasts	1 p.p.m. (light)/ 100 p.p.m. (dark)		74
	BALB/3T3	15 p.p.m. (light)	No dark experiments	78
Malonic acid derivative	Human cervix cells	30 p.p.m. (light) >100 p.p.m.		75
Ammonium salt	Cortical cells	7 p.p.m.	Uncontrolled light	86

[a]LC_{50} is defined as the dose at which 50% of the cells die.

Animal studies of fullerenes have found minimal dermal and oral toxicity, but more pronounced acute toxicity upon intravenous administration[76–79]. The LD_{50} for one type of water-soluble fullerene was found to be 600 mg/kg body weight from direct intraperitoneal injection[80], and an unusual form of kidney damage was observed at lower doses[80,81]. This literature illustrates that water-soluble fullerenes are active biological materials; however, the specific surface treatment and illumination conditions of fullerene species are critical for determining their quantitative toxicological response *in vitro* (**Table 1**).

The first peer-reviewed toxicological studies of well-characterized, single-walled carbon nanotubes (SWNT) are now in the press; two separate groups have independently reported the development of granulomas when the nanotubes are instilled in rat and mouse lungs at concentrations of 1–5 mg/kg[82,83]. This histology is different from the inflammatory responses generated by larger carbon fibers and quartz particles. It can occur when the body has a foreign-body response to materials and is indicated in individuals with tuberculosis as well as those exposed to beryllium dust. It is notable that such histology has also been observed at the sites of wear debris in replacement joints[84].

The interpretation of these data is controversial[85]. Granulomas are not commonly observed in pulmonary toxicology and their medical significance has not been established. Also, it is unclear whether the responses observed arose from the SWNTs or from the associated metal catalyst particles present in the samples. Additionally, choosing the appropriate dose for lung instillation experiments is challenging in the absence of data on the respirable levels of single-walled carbon nanotubes in air. Even so, the studies represent the earliest attempts to answer difficult and important questions, and will encourage more collaborative work between toxicology and nanotechnology researchers.

Conclusions

In this new century, emerging technologies face a more skeptical and demanding public—not only must their benefits to society be clear, but scientists and engineers must also anticipate and characterize potential risks associated with their implementation. In the case of nanotechnology, these risks surround the potential environmental implications of the widespread use of engineered nanomaterials. Currently, nanomaterial exposures and health effects are unlikely to pose any substantial risk to public health given the most prevalent exposure routes and the limited scope of their use. However, as the quantity and types of engineered nanomaterials used in society increases, the potential for unintended environmental consequences will also increase. Though it is challenging to assess the risks of engineered nanomaterials before commercial products are well defined, proactive research is critical to ensuring a sustainable nanotechnology industry.

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Erratum: Erbitux diagnostic latest adjunct to cancer therapy

Peter Mitchell
Nat. Biotechnol. **22, 363–364 (2004)**

On page 363, column 2, line 14, sepsis is incorrectly listed as one of the side effects of Erbitux. The sentence should have read "potential side effects such as rare cases of interstitial lung disease."

Corrigendum: Correction of multi-gene deficiency *in vivo* using a single 'self-cleaving' 2A peptide–based retroviral vector

Andrea L Szymczak, Creg J Workman, Yao Wang, Kate M Vignali, Smaroula Dilioglou, Elio F Vanin & Dario AA Vignali
Nat. Biotechnol. **22, 589–594 (2004)**

In Figure 3b, the labels for CD3e and CD3e-2A were inverted. Thus, the second band should have been labeled CD3e-2A and the third band labeled CD3e.

Corrigendum: The potential environmental impact of engineered nanomaterials

Vicki L Colvin
Nat. Biotechnol. **21, 1166–1170 (2003)**

In the Acknowlegments, line 3, the name Cafer Yavuz was misspelled as Cafer Tevuyz.

Addendum: Correction of multi-gene deficiency *in vivo* using a single 'self-cleaving' 2A peptide–based retroviral vector

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Technical note: Recent experiments with other 2A-linked constructs have suggested that cleavage efficiency can be influenced by the protein NH_2-terminal to the 2A peptide. In some instances, we have found that cleavage efficiency can be improved by placing a Gly-Ser-Gly linker between NH_2-terminal protein and the 2A peptide.